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Differential Inhibition of Transforming Growth Factor β1 and β2 Activity by α_2-Macroglobulin*

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Affinity labeling and immunoprecipitation studies demonstrate that α_2-macroglobulin (α_2M) is the major serum-binding protein for transforming growth factors β1 and β2 (TGF-β1 and TGF-β2). Purified α_2M inhibits the binding of both ^{125}I-TGF-β1 and ^{125}I-TGF-β2 to cell surface receptors at I_{50} values of 200 and 10 μg/ml, respectively. α_2M (200 μg/ml) does not block TGF-β1 inhibition of CCL-64 mink lung cell growth but reduces this activity of TGF-β2 10-fold. The electrophoretic migration of ^{125}I-TGF-β·α_2M complexes on polyacrylamide gels under nondenaturing conditions demonstrates that α_2M has 10-fold greater affinity for TGF-β2 than for TGF-β1. Each of these complexes comigrates as a single band with the fast form of α_2M. We suggest that α_2M is an important differential regulator of the biological activities of TGF-β1 and TGF-β2 *in vivo*.

Transforming growth factors β1 and β2 (TGF-β1 and TGF-β2),[1] two multifunctional modulators of cell growth, differentiation, and numerous other cellular processes, are each highly conserved 25-kDa homodimers that share 71% sequence homology (1). Although in most cases TGF-β1 and TGF-β2 share similar or identical receptor-binding properties and are indistinguishable in most biological assays (2), recent reports suggest that they may each be involved in distinct physiological processes (3–8).

TGF-β1 and TGF-β2 secreted from cells in culture and TGF-β1 released from platelets are biologically latent (7, 9–12) and as such cannot bind to TGF-β receptors (7, 11, 12). However, these latent forms can be readily activated by transient acidification (pH 3, 5 min) (7, 9–12). For platelet TGF-β1, this latency is conferred by noncovalent association of mature TGF-β1 with the remainder of its precursor sequence (74 kDa) and a third 135-kDa protein called the modulator (11, 12); the structure of secreted latent TGF-β2 is not known. A second type of latent TGF-β1, accounting for almost all serum latent TGF-β1, consists of a complex of mature TGF-β1 with α_2-macroglobulin (α_2M) (13, 14). α_2M is a plasma or serum-derived inhibitor of proteases (15) and several bioactive peptides (13, 16–18) and serves in the clearance of proteins from the circulation via endocytosis by target α_2M receptor-positive cells (19). α_2M exists as two distinct tetramers, designated slow and fast, distinguished by their relative electrophoretic mobility through polyacrylamide gels under nondenaturing conditions (20). Slow α_2M is available for binding proteases and after such binding is converted to the fast form, which is the clearance form (19, 20). The TGF-β1·α_2M complex migrates as the fast form of α_2M (14), suggesting that TGF-β1·α_2M is a clearance complex.

Bioavailability of active TGF-β1 and TGF-β2 *in vivo* depends on the balance of secretion of latent complexes by cells, activation of these complexes, as well as inactivation and clearance of the active TGF-βs. Any one of these processes is a potential site for differential regulation of TGF-β1 and TGF-β2 extracellular signaling. In this report, we demonstrate that these two forms of TGF-β are differentially inactivated by α_2M and provide a mechanism by which this may occur.

MATERIALS AND METHODS

Iodination of TGF-β1 and TGF-β2—Porcine TGF-β1 and -β2 (R and D Systems, Minneapolis) were radioiodinated using Enzymobeads as described previously (7), except the iodination reaction time was 5 min, and the beads were resuspended by gentle vortexing once every min during this period. Both TGF-βs iodinated equally well, with specific activities ranging from 2.8 to 3.2 μCi/pmol. After iodination, the biological activity of TGF-β1 as measured by a CCL-64 mink lung cell growth inhibition assay (7) was unaltered, whereas that of TGF-β2 increased 2.3-fold.

Radioreceptor Assay for TGF-β1 and TGF-β2—The ability of serum and purified bovine plasma α_2M (Boehringer Mannheim) to inhibit the binding of ^{125}I-TGF-β1 and -β2 to receptors on A549 cells was determined using methods described for the inhibition of ^{125}I-TGF-β1 binding to receptors by anti-TGF-β antibodies (21).

Effect of α_2M on Biological Activity of TGF-βs—The effect of purified human α_2M and bovine sera on the biological activity of TGF-β1 and TGF-β2 was determined using a CCL-64 mink lung cell growth inhibition assay (7). CCL-64 mink lung cells from a subconfluent flask were transferred to Dulbecco's modified Eagle's medium containing 0.2% fetal bovine serum and seeded in 24-well Costar dishes at 5×10^4 cells/0.5 ml/well. After 1 h at 37 °C for cell attachment, TGF-βs, α_2M, and sera were added. After 22 h, cells were pulsed with 0.25 μCi of 5′-[^{125}I]Iodo-2′-deoxyuridine (^{125}I-UdR) for 2 h, and cells were then fixed by the addition of 1 ml of acetic acid/methanol (1:3, v/v). After a minimum of 1 h at room temperature, the wells were washed twice with 2 ml of 80% methanol. The label was then extracted with 1 N NaOH for 30 min at room temperature.

Formation of TGF-β·α_2M Complexes—TGF-β·α_2M complexes were either cross-linked and separated by a 3–10% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described (13) or not cross-linked and measured using a 5% native PAGE (14). For SDS-PAGE, the TGF-β complexes were formed by incubation of 100 pM ^{125}I-TGF-βs with 2.5% human serum or 75 μg/ml purified bovine plasma α_2M at room temperature for 2 h in phosphate-buffered saline (PBS), pH 7.4. Cross-linking was achieved at 4 °C by the addition of 0.20 volume of 5 mM bis(sulfosuccinimidyl)suberate, and the reaction was stopped by the addition of

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[1] The abbreviations used are: TGF, transforming growth factor; α_2M, α_2-macroglobulin. SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; CS, calf serum; FBS, fetal bovine serum.

0.05 volume of 2.5 M glycine, as described (13). Samples then received an equal volume of SDS-PAGE sample loading buffer (0.0025% bromphenol blue, 10.5% glycerol, 2% SDS, and 60 mM Tris-HCl, pH 6.8) and were heated for 5 min at 100 °C. For native PAGE, the ^{125}I-TGF-β·α2M complexes were formed and electrophoresed as described for TGF-β1 (14). Briefly, α2M (0, 0.12, 0.37, 1.1, 3.3, and 10 μg) was incubated with 1 ng of ^{125}I-TGF-β in 0.1 ml of 0.1 M sodium phosphate (pH 7.0) containing 0.1% crystalline bovine serum albumin at 22 °C for 1 h. An equal volume of loading buffer (20% glycerol, 41 mM Tris, 40 mM sodium borate, pH 8.6) was added, and 30 μl of this mixture was electrophoresed at 4 °C through a 5% native polyacrylamide gel, pH 5.7, with a 4% stacking gel, pH 6.1, as described (14).

Immunoprecipitation of TGF-β·α2M Complexes—2.5% human serum in PBS was incubated with either 50 pM ^{125}I-TGF-β1 or ^{125}I-TGF-β2 for 2 h at room temperature. These complexes were then cross-linked with bis(sulfosuccinimidyl)suberate as before, and then 200-μl aliquots were incubated overnight at 4 °C with either 200 μg of monospecific rabbit IgG against human α2M (Sigma), 200 μg of rabbit anti-TGF-β IgG, or 200 μg of preimmune rabbit IgG adjusted to a final volume of 300 μl with PBS. Antibody-protein complexes were precipitated with 200 μl of 10% fixed Staph A cells (Boehringer Mannheim) in IP buffer (22) by rocking for 1.5 h at room temperature, and cells were washed three times with 1 ml of IP buffer. The washed cell pellet was boiled for 5 min in 400 μl of SDS-PAGE loading buffer, and 100 μl of the 13,000 × *g* supernatants was analyzed by 3–10% gradient SDS-PAGE as before (13).

Enzyme Immunoassay for Bovine α2M—Nunc Maxisorb microtiter wells (microplate 1) were coated with 200 ng of pure bovine plasma α2M in 100 μl/well of PBS, 0.02% NaN_3. After overnight incubation at 4 °C, excess buffer was discarded, and wells were blocked with 250 μl of 1% bovine serum albumin (crystalline) in PBS, 0.02% NaN_3 for about 3 h at room temperature. During this incubation, monospecific rabbit anti-human α2M (750 ng/ml) in PBS containing 1% bovine serum albumin (crystalline), 0.05% Tween 20, and 0.02% NaN_3 was preincubated in a second microtiter plate (microplate 2) with various concentrations of standard pure bovine α2M, calf serum, or fetal bovine serum for 2 h at room temperature. Microplate 1 was then washed four times with WB (PBS, 0.05% Tween 20). Then, 100-μl aliquots of the samples in microplate 2 were transferred to microplate 1. Following a 1-h incubation at room temperature with shaking, wells (microplate 1) were washed seven times with WB and coated with 100 μl of phosphatase-linked goat anti-rabbit IgG (Kirkegaard and Perry, Gaithersburg, MD) in PBS containing 1% bovine serum albumin (crystalline), 0.05% Tween 20, and 0.02% NaN_3, and wells were incubated and washed as in the previous step and further washed twice with distilled water. Phosphatase substrate, 100 μl (1 mg/ml *p*-nitrophenyl phosphate in diethanolamine buffer; Kirkegaard and Perry), was added to each well, and the reaction was allowed to proceed for 75 min at room temperature. The difference in absorbance at 410 and 450 was measured with a Dynatech MR600 enzyme-linked immunosorbent assay reader (Chantilly, VA).

RESULTS AND DISCUSSION

During tissue injury, TGF-β1 released from platelets as a biologically latent complex is activated by dissociation from this complex (11) via a mechanism that is not fully understood. α2M, the major serum-binding protein of TGF-β1, has been postulated to inactivate excess active TGF-β1, thereby preventing the direct action of TGF-β1 distant from the site of injury (13, 14). We have been interested in identifying other binding proteins that may differentially inactivate TGF-β1 and TGF-β2 in the circulation or in tissues. Initially, we assessed the ability of calf, fetal bovine, rabbit, and human sera to inhibit the binding of ^{125}I-TGF-β1 and ^{125}I-TGF-β2 to A549 human lung carcinoma cells. We found that all these sera inhibited the binding of ^{125}I-TGF-β2 far more than the binding of ^{125}I-TGF-β1 (Table I).

In order to understand the mechanism for the serum-dependent differential inhibition of TGF-β receptor binding, we identified serum-binding proteins for TGF-β1 and TGF-β2 by affinity labeling methods (13). The radiolabeled TGF-βs were incubated with 2.5% human serum or 75 μg/ml purified bovine plasma α2M for 2 h at room temperature, and these samples were either briefly cross-linked with bis(sulfosuccinimidyl)suberate or not cross-linked, boiled in SDS loading buffer with or without dithiothreitol, and then analyzed autoradiographically after separation by 3–10% gradient SDS-PAGE (Fig. 1). In the absence of dithiothreitol (Fig. 1*A*), each of the two radiolabeled TGF-βs cross-linked to a dimeric α2M (300-kDa) reference marker, as well as a 300-kDa moiety in serum, with no significant cross-linking to other components. In the presence of dithiothreitol (Fig. 1*B*), all these complexes were shifted to an apparent molecular mass of about 180 kDa. The majority of these complexes (Fig. 1, *A* and *B*) were noncovalent because, in the absence of the cross-linker, they were completely dissociated by boiling in SDS. These results agree with those of a previous report on TGF-β1·α2M complex (13) and further indicate that α2M is also the major serum-binding protein for TGF-β2.

TABLE I

Binding of TGF-β1 and TGF-β2 to A549 cells is differentially blocked by serum

The ability of serum to block the binding of TGF-β types 1 and 2 to A549 human lung carcinoma cells was assayed as described (11). ^{125}I-TGF-βs (50 pM) were incubated with sera for 2 h at room temperature prior to receptor binding. Results represent the average of three determinations with S.D. < 10% of the mean.

Serum (10%)	^{125}I-TGF-β1		^{125}I-TGF-β2	
	cpm	*% of control*	*cpm*	*% of control*
None	3890	100	2930	100
Calf	3110	80	1020	35
Fetal bovine	3810	98	2200	75
Rabbit	2370	61	570	19
Human	2410	62	540	18

For further verification that α2M itself, rather than another protein with a similar apparent molecular mass is the major serum-binding protein for TGF-β2, human serum was first affinity labeled with ^{125}I-TGF-βs, and the complexes were immunoprecipitated with either monospecific anti-α2M IgG, anti-TGF-β IgG, or preimmune IgG. These complexes were then analyzed autoradiographically after separation by 3–10% gradient SDS-PAGE (Fig. 2). When compared with the non-immunoprecipitated complexes (Fig. 2, *lanes 1* and *5*), anti-α2M IgG immunoprecipitated most of the 300-kDa complex and virtually no free TGF-βs (20 kDa; *lanes 2* and *6*). Anti-TGF-β, on the other hand, immunoprecipitated only the non-cross-linked TGF-βs. Control preimmune IgG precipitated neither free TGF-βs nor their corresponding 300-kDa complexes. This experiment demonstrates that α2M is the major serum-binding protein for TGF-β2 as for TGF-β1. Since anti-TGF-β IgG did not immunoprecipitate the TGF-β2·α2M complexes, it appears that α2M binds to TGF-β2 by a molecular trapping mechanism similar to that described previously for several proteases (15) and TGF-β1 (13).

After showing that α2M is the major serum-binding protein for both TGF-β1 and TGF-β2, we determined whether the differential inhibition of TGF-β1 and TGF-β2 receptor binding by serum (Table I) could be explained by their binding to α2M. ^{125}I-TGF-β1 and ^{125}I-TGF-β2 were each incubated with various concentrations of purified α2M, and the ability to inhibit receptor binding was measured, using A549 human carcinoma cells. As shown in Fig. 3, although the receptor binding of each TGF-β was inhibited by purified α2M, the effective concentration of α2M required to inhibit TGF-β2 receptor binding was 5% of that for the inhibition of TGF-β1 receptor binding. These results indicate that the differential inhibition of TGF-β1 and TGF-β2 receptor binding by sera (Table I) could be attributable to α2M. α2M did not inhibit TGF-β receptor binding when cells were first preincubated with α2M and excess α2M washed away before labeling the

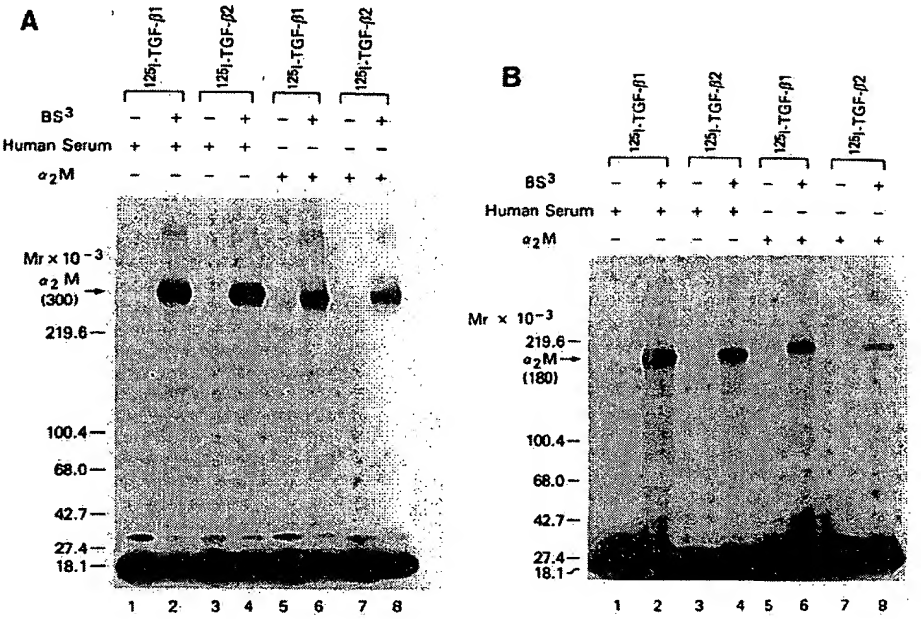

FIG. 1. **Affinity labeling of human serum and bovine α_2M with ^{125}I-TGF-β1 or ^{125}I-TGF-β2.** Human serum (*lanes 1–4*) and purified α_2M (*lanes 5–8*) were incubated with either 100 pM ^{125}I-TGF-β1 (*lanes 1, 2, 5, 6*) or 100 pM ^{125}I-TGF-β2 (*lanes 3, 4, 7, 8*) for 2 h at room temperature. Samples in *lanes 2, 4, 6,* and *8* were cross-linked as described under "Materials and Methods." These samples were treated (100 °C, 5 min) with SDS-PAGE loading buffer either in the absence of reducing agent (*panel A*) or in the presence of 100 mM dithiothreitol (*panel B*) and then electrophoresed through 3–10% gradient SDS-polyacrylamide gels. *BS*^{3} bis(sulfosuccinimidyl)suberate.

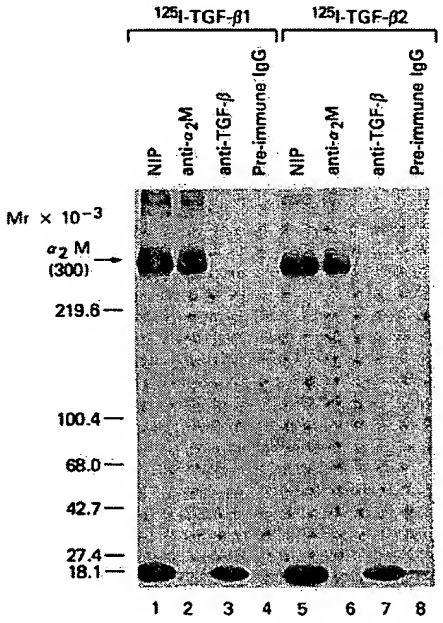

FIG. 2. **Immunoprecipitation of TGF-β1 and TGF-β2 serum-binding proteins.** Human serum (2.5%) was incubated with either 100 pM ^{125}I-TGF-β1 (*lanes 1–4*) or ^{125}I-TGF-β1 (*lanes 5–8*) for 2 h at room temperature and cross-linked with 1 mM bis(sulfosuccinimidyl)suberate for 2 min at 4 °C. The cross-linked proteins were immunoprecipitated with either anti-human α_2M IgG (*lanes 2* and *6*), anti-TGF-β IgG (*lanes 3* and *7*) or preimmune IgG (*lanes 4* and *8*), as described under "Materials and Methods." *Lanes 1* and *5* (*NIP*) represent the nonimmunoprecipitated samples. Immunoprecipitates were electrophoresed through a 3–10% gradient SDS-polyacrylamide gel.

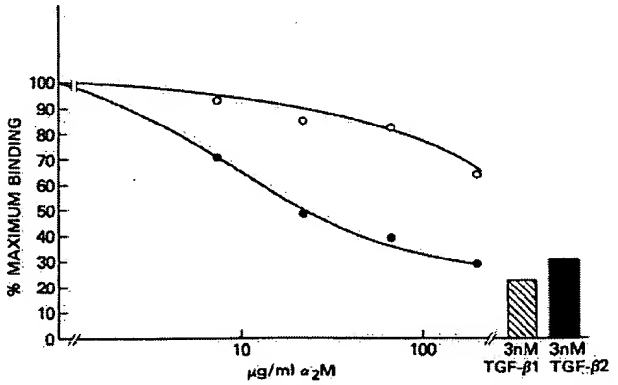

FIG. 3. **Inhibition of the binding of ^{125}I-TGF-β1 (*open circles*) and ^{125}I-TGF-β2 (*closed circles*) to A549 human lung carcinoma cells by α_2M.** Radiolabeled ligands, 50 pM each, were first incubated with α_2M for 2 h at room temperature, and then binding of these ligands to A549 cells was measured as described under "Materials and Methods." Either unlabeled TGF-β1 or TGF-β2 (3 nM) (*hatched* and *solid bars*, respectively) was used as a competitor of its respective radiolabeled ligands to determine nonspecific binding. Data are presented as percent of total binding (^{125}I-TGF-βs added only), which were 2920 and 2150 cpm for ^{125}I-TGF-β1 and ^{125}I-TGF-β2, respectively.

cells with the ^{125}I-TGF-βs, demonstrating that α_2M does not modify the ability of TGF-β receptors to bind ^{125}I-TGF-βs. This differential inhibition of receptor binding could also not be explained by TGF-β2 having lower affinity for receptors than TGF-β1 because the K_d values for TGF-β1 and TGF-β2 receptor binding on these cells, measured to be 2.3 and 3.6 pM, respectively (3), are not sufficiently different. Thus, the differential inhibition of receptor binding results from differential inactivation of TGF-β1 and TGF-β2.

We next asked whether α_2M would also differentially inhibit the biological activities of these TGF-βs in a manner consistent with its inhibition of receptor binding. For this purpose a rapid and sensitive growth inhibition assay using CCL-64 mink lung epithelial cells was employed (7). TGF-β1 and TGF-β2 were assayed for biological activity either in the absence or presence of 200 μg/ml pure bovine α_2M (Fig. 4). α_2M reduced the apparent biological activity of TGF-β2 to 10% of nontreated controls, although it did not modify the biological activity of TGF-β1. Inability of purified α_2M to

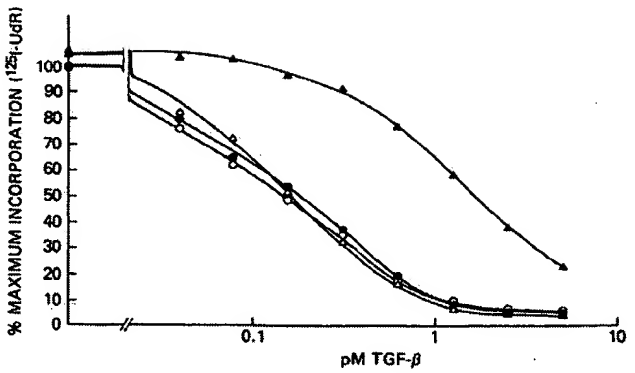

FIG. 4. **Effect of α_2M on the ability of TGF-β1 and TGF-β2 to inhibit growth of CCL-64 mink lung cells.** Growth was measured by the incorporation of ^{125}I-UdR into DNA. TGF-β1 was assayed either alone (*open circles*) or with 200 μg/ml α_2M (*open triangles*). Likewise, TGF-β2 was assayed either alone (*closed circles*) or with 200 μg/ml α_2M (*closed triangles*). Data are presented as percent incorporation of control (without the addition of TGF-β or α_2M; cpm of control = 2750). All points represent the average of duplicate determinations with S.D. < 5% of the mean. *UdR*, 5′-[^{125}I] Iodo-2′-deoxyuridine.

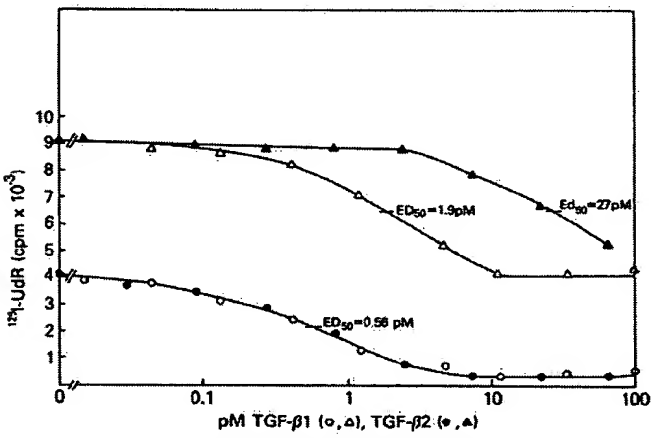

FIG. 5. **Effect of calf serum on the ability of TGF-β1 and TGF-β2 to inhibit growth of CCL-64 mink lung cells.** Growth was measured by the incorporation of ^{125}I-UdR into DNA. TGF-β1 was assayed either alone (*open circles*) or with 10% CS (*open triangles*). Likewise, TGF-β2 was assayed either alone (*closed circles*) or with 10% CS (*closed triangles*). All points represent the average of duplicate determinations with S.D. < 5% of the mean. *UdR*, 5′-[^{125}I] Iodo-2′-deoxyuridine.

block the biological activity of TGF-β1 has been reported previously (13). Consistent with this differential blocking of growth inhibition, 10% calf serum (CS) reduced the specific activities of TGF-β1 and TGF-β2 on CCL-64 cells by 3.4-fold and 48-fold, respectively (Fig. 5), thereby reducing the biological activity of TGF-β2 14.2-fold over that of TGF-β1. Fetal bovine serum (FBS), 10%, counteracted the growth inhibition of TGF-β2 by only 3.2-fold over that of TGF-β1 (data not shown). This small inhibition of TGF-β2 by FBS is nevertheless consistent with the marginal inhibition of TGF-β2 receptor binding by FBS (Table I).

The α_2M content of the sera used in the above study was measured using a competitive inhibition enzyme-linked immunosorbent assay (Fig. 6) in order to determine whether the relative inactivation of TGF-β1 and TGF-β2 reflected the quantity of α_2M in sera. The relative levels of α_2M in CS (6.9 mg/ml) and FBS (0.8 mg/ml) measured by this assay could

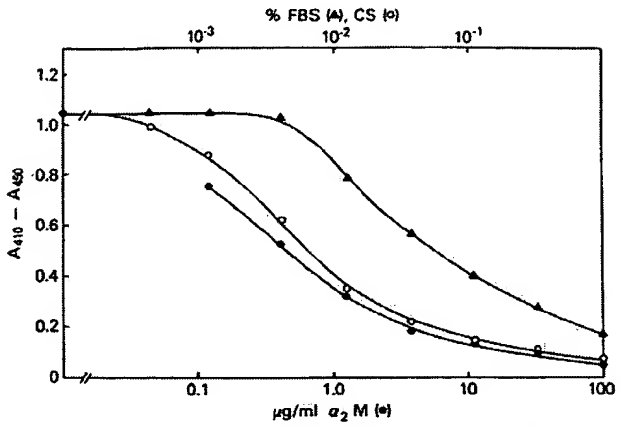

FIG. 6. **Quantitation of α_2M in calf serum and fetal bovine serum by a competitive inhibition enzyme-linked immunosorbent assay.** The concentrations of α_2M in CS (*open circles*) and FCS (*closed triangles*) were measured by their ability to block the binding of monospecific rabbit anti-human α_2M IgG to pure bovine plasma α_2M bound to microtiter wells. Pure bovine plasma α_2M (*closed circles*), used as a standard, blocked >95% antibody binding at >100 μg/ml, with an ED$_{50}$ of 0.45 μg/ml. All points represent the average of duplicate determinations with S.D. < 10% of the mean. The assay was conducted as described under "Material and Methods."

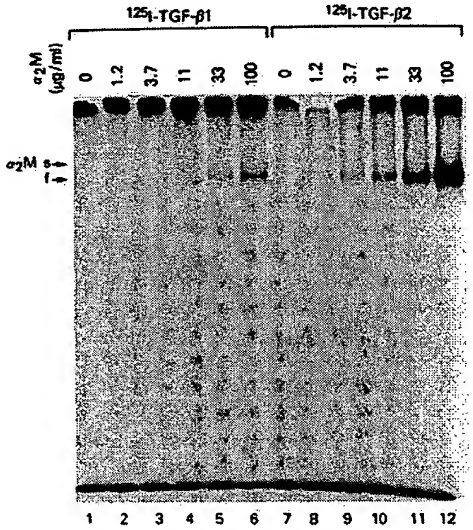

FIG. 7. **Electrophoretic migration of ^{125}I-TGF-β1·α_2M (*lanes 2–6*) and ^{125}I-TGF-β2·α_2M (*lanes 8–12*) through a 5% polyacrylamide gel under nondenaturing conditions.** The radiolabeled ligands (400 pM) were incubated for 1 h at room temperature in buffer alone (*lanes 1* and *7*) or with various concentration of α_2M, as indicated above, and then electrophoresed at 4 °C, as described under "Materials and Methods." *Arrows* on the *left* indicate migration of slow (*s*) and fast (*f*) forms of bovine plasma α_2M.

account for these differences. Our measurement of α_2M in three different lots of both CS and FBS from GIBCO indicates that the levels of α_2M are at least 7-fold higher in CS than in FBS. These results have important implications *in vitro*, since they demonstrate that the relative specific activities of TGF-β1 and TGF-β2 on cells are different when measured in the presence of either CS, FBS, or under serum-free conditions. Thus, in the presence of serum, the biological activity of TGF-β2, with respect to TGF-β1, tends to be underestimated, depending on the level of α_2M in the particular batch of serum. Our results indicate that assessment of the inherent

relative responsiveness of a cell to different TGF-β subtypes should be conducted under α_2M-free conditions, in which the concentrations of active TGF-βs are not differentially modified.

In order to understand the mechanism by which α_2M differentially inhibits the activities of these two TGF-βs, we measured the relative affinities of TGF-β1 and TGF-β2 for α_2M by following the electrophoretic migration of the non-cross-linked complexes through 5% polyacrylamide gels under nondenaturing conditions (Fig. 7). Cross-linking was avoided when measuring relative affinities for α_2M because of a potential difference in the efficiency of the two TGF-βs to cross-link to α_2M. Densitometric analysis of the TGF-β·α_2M complexes on the native gel showed that approximately 10-fold more α_2M was required to achieve the same degree of complex formation with TGF-β1 than with TGF-β2, indicating that α_2M had about a 10-fold greater affinity for TGF-β2. This suggests that the mechanism for the greater inactivation by TGF-β2 of bioactivity by α_2M may be due to increased complex formation. For both TGF-βs, binding occurred exclusively to the fast form of α_2M, consistent with the previous report on TGF-β1·α_2M (14). Whether these TGF-βs bind to fast α_2M or bind to slow α_2M which is then rapidly converted to fast α_2M is not known, although evidence supports the former model for TGF-β1 (14).

Although the mechanism behind the clearance of TGF-β from the circulation is as yet not resolved, evidence supporting the hypothesis that TGF-β is cleared via the formation of a complex with α_2M is as follows. (*a*) TGF-β·α_2M complexes migrate through 5% native PAGE as the fast or clearance form of α_2M. (*b*) Liver is the major route of clearance for both intravenously administered TGF-β1 (23) and plasma fast α_2M (24). (*c*) The clearance half-lives of intravenously administered TGF-β1 (23), TGF-β2,[2] and fast α_2M (25), measured in adult male rats, are all approximately 2.5 min. Because TGF-β1 and TGF-β2 are both cleared at approximately the same rate, we conclude that if TGF-βs are cleared predominantly through α_2M receptors then the binding of TGF-βs to α_2M cannot be rate limiting for their clearance from plasma.

Recent studies demonstrating secretion of α_2M from a variety of non-hepatic cells (26–29) and the presence of α_2M receptors on many different cell types (30–32) provide strong evidence that α_2M functions locally within tissue interstitial spaces. If so, α_2M in extracellular matrices may act as a differential regulator of the biological activities of TGF-β1 and TGF-β2 secreted by neighboring cells. In this scenario, α_2M would function as a selective screen through which active TGF-β1 would penetrate better than active TGF-β2. By this means, preferential induction of active TGF-β2 over active TGF-β1, such as recently reported for retinoic acid or Ca^{2+} treatment of keratinocytes (8, 33), would restrict the spatial range of TGF-β action from autocrine/paracrine to more autocrine. In this manner, the selective induction of each of these two TGF-βs may allow the differential regulation of certain unique and critical tissue functions. The possibility that TGF-β·α_2M complexes are cleared by α_2M receptors in tissues, a mechanism that may potentiate the preferential inactivation of TGF-β2, is presently under investigation.

[2] D. Danielpour and M. B. Sporn, unpublished data.

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